essential regulatory protein cofilin severs actin filaments, which accelerates network remodeling by increasing the concentration of filament ends available for elongation and subunit exchange. Although cofilin effects on actin filament assembly dynamics have been extensively studied, the molecular mechanism of cofilin-induced filament severing is not understood. Here we demonstrate that actin filament severing by vertebrate cofilin is driven by the linked dissociation of a single cation per bound cofilin, which alters filament mechanical properties. Vertebrate cofilin only weakly severs Saccharomyces cerevisiae actin filaments lacking this ''stiffness cation'' unless a stiffness cation-binding site is engineered into the actin molecule. Vertebrate cofilin rescues the viability of a Saccharomyces cerevisiae cofilin deletion mutant only when the stiffness cation site is simultaneously introduced into actin, demonstrating that filament severing is the essential function of cofilin in cells. The work presented here reveals that site-specific interactions with cations serve a key regulatory function in actin filament fragmentation and dynamics.

119-Plat
How Actin Initiates the Motor Activity of Myosin
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Fundamental to cellular processes are directional movements driven by molecular motors. A common theme for these and other molecular machines driven by ATP is that controlled release of hydrolysis products is essential to use the chemical energy efficiently. Mechanochemical transduction by myosin motors on actin is coupled to unknown structural changes that result in the sequential release of inorganic phosphate (Pi) and MgADP. We will describe how key regions of the motor play a role at the actin interface to trigger different transitions during the powerstroke. We will also present a myosin structure that explains how actin initiates force generation and movement. This structure possesses an actin interface that differs from previously seen pre- and post-stroke states of the motor. The structure also describe the tunnel (back door) that creates an escape route for Pi with a minimal rotation of the myosin lever arm that drives movements. Functional studies allow us to propose that this state represents the beginning of the powerstroke on actin, and that Pi translocation from the nucleotide pocket triggered by actin binding initiates force generation by myosin. This elucidates a strategy that may be common to many molecular machines.

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Structural Basis for Calcium Regulation of the Motor Function of Myosin-5a
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The motor function of vertebrate myosin-5a (Myo5a) is regulated by its globular tail domain (GTD) in a Ca^{2+}-dependent manner. We recently demonstrated that the CaM bound to the first IQ motif (IQ1) of Myo5a is necessary for Ca^{2+}-dependent regulation of Myo5a motor function and proposed that Ca^{2+} induces the conformational change of the CaM in IQ1 and prevents the interaction between the head and the GTD, thus activating the motor function. However little is known about the Ca^{2+}-induced structural change of Myo5a. Here we report a crystal structure of truncated Myo5a containing the motor domain and IQ1 (MD-IQ1) in complex with Ca^{2+}-bound calmodulin (Ca^{2+}-CaM) at 2.5 Å resolution. Compared with M5a-IQ1/apo-CaM structure, M5a-IQ1/Ca^{2+}-CaM displays a large conformational change in CaM/IQ1 but little difference in the motor domain. Ca^{2+}-binding induces N-lobe and C-lobe of CaM to transform from closed-conformation and semi-open conformation to an open conformation and flip-flop of CaM relative to IQ1 motif. Our work presents the first atomic-resolution structure of Myo5a in complex with Ca^{2+}-CaM and provides the structural insight into the mechanism of Ca^{2+} regulation of Myo5a function.

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Structural Determinants of Myosin I Mechanosensing: The N Terminal Region
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Myosin-I molecular motors are mechanically and kinetically tuned to function in a host of cellular processes. The myosin-I family members Myosin-IB (Myo1b) and Myosin-IC (Myo1c) have similar structures and ATPase kinetics; however, they display very different behaviors when mechanical loads are placed on the motors. The ATP-dependent, actin-detachment kinetics of Myo1c are not significantly affected by loads that resist the myosin power-stroke of <2 pN, allowing the myosin to generate power and act as a molecular transporter. In contrast, similar loads slow the ATP-dependent, actin-detachment kinetics of Myo1b by two-orders of magnitude, enabling the myosin to function as a tension-sensing anchor. We recently determined the crystal structure of the Myo1b motor domain and found that the N-terminal region (NTR) is in a conformation not seen in other myosin structures. Because the NTR sequence is highly variable and alternatively spliced within the myosin-I family, we investigated the role of the NTR in tuning the mechanochemical properties of myosin-I. We expressed recombinant constructs of Myo1b and Myo1c that had their NTRs deleted or swapped, and we characterized their kinetic and mechanochemical properties using in vitro motility assays, stopped-flow techniques, and single molecule optical trapping techniques. Our results show that the NTR plays an important role in tuning the mechanochemical properties of Myo1b and Myo1c, however the effects of this tuning are very different between these isoforms. These differences in the role of the NTR suggest that the sequence diversity and alternative splicing of this region may play a vital role in generating diversity of myosin-I function in the cell. This work was supported by grants from the NIH (GM057247 to E.M.O. and HL123623 to M.J.G.).

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Myosin 5 Side Steps along Actin
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Myosin 5a is a dual-headed molecular motor fuelled by ATP hydrolysis that transports cargo along actin filaments. Although the kinetic cycle is well understood, there is inconclusive and conflicting information on the motion that describes individual steps. Studies aimed at revealing the stepping mechanism have either reported periods of increased flexibility or proposed partitioning of the step into one, or multiple sub-events. All resulting models involve a forward aiming power stroke followed by a Brownian search mechanism. We used interferometric scattering microscopy to reveal the motion of the heads with simultaneous nanometer spatial and millisecond temporal precision during processive motion. We observed a single, spatially constrained transient state with a 17.5 ± 0.6 ms exponential lifetime from which myosin reaches the desired binding site in a highly controlled and efficient manner. The position and dynamics of the transient state demonstrate a significant torsional component to the power stroke. Our results show how the motor restricts the motion of the unbound heads during stepping to optimise the probability of finding the next binding site and conserve the 74 nm step size.

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Probing Lipid Vesicle Transport in 3D by Teams of Myosin Va Motors at Suspended Actin Intersections in vitro
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In cells, myosin Va motor teams are forced to transport vesicles through a complex three-dimensional (3D) actin meshwork. To characterize how these motors navigate this physically challenging meshwork, we created actin filament intersections adhered to a coverslip surface and confronted a team of ~10 myosin Va HMM motors carrying a 350nm synthetic lipid vesicle. When approaching the intersection on the lower filament, the motor-vesicle complex (MVC) switched to the upper intersecting filament with a 51% probability while crossing over the intersection with only a 33% probability. If approaching the intersection on the upper filament, the MVC preferred staying on the upper filament through the intersection and only switched to the lower filament with a 31% probability. These data suggest that the extent of MVC surface contact with the target binding zone of the intersecting actin filaments dictates the directional outcome. To build complexity, actin filaments were strung between 3μm beads, creating suspended filament intersections. The 3D spatial relations between the MVC and the individual filaments were determined by super-resolution STORM imaging. As the MVC approached the intersection, the complex spiraled around the actin